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The recovery rate of Simental spermatozoa frozen of post thawing by using tris dilution with different egg yolks

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Abstract. The yolk content of each poultry varies so that the quality of spermatozoa diluents from various egg yolks will also vary. The purpose of this study was to determine the effect of the use of chicken, duck, and egg yolks-quail in Tris dilution to the quality of Simental spermatozoa. Semen used in this study was semen Simental bulls from at the Tuah Sakato Artificial Insemination center, Payakumbuh, West Sumatera. The research used a completely randomized design (CRD) of three treatments with ten replications. The results of the research showed that the use of different egg yolk diluent gave a significant effect in motility, viability, membrane plasma intact, recovery rate and abnormalities spermatozoa Simental. Conclusion, the duck egg yolk to the tris diluent for Simental bulls spermatozoa preservation is the best diluent with high motility (43.00%), high intact plasma membrane (54.67%), high the viability (45.17%) with low abnormality spermatozoa (13.33%) and lower recovery rate (57.33 %) compared to the tris egg yolk-chicken diluent and tris egg yolk-quail.

1. Introduction

The Simental cattle are meat and milk-producing dual-purpose cattle. Simental cows have a higher body weight than local cows so that Simental cows have become an idol among farmers. This condition made the demand for Simental beef frozen semen increase from other semen at several Artificial Insemination Centers.

The diluent commonly used in some Artificial Insemination Centers is tris fructose. Each diluent has advantages and disadvantages but the most important thing is to be able to maintain the quality of spermatozoa during the dilution, freezing and thawing again after freezing. the diluent must be able to provide food as an energy source for spermatozoa, protect spermatozoa from cold shock (cold shock), function as a buffer or buffer to prevent changes in pH [1]. The cold shock causes changes in membrane lipid arrangement due to changes in the lipid phase and membrane fluidity in spermatozoa of cattle[2].

One of the diluents added in tris is egg yolk. Egg yolks contain lecithin and phospholipid, besides that egg yolks can also contribute nutrients to spermatozoain the form of carbohydrates, proteins and some vitamins [3], very useful in protecting the membrane of spermatozoa during cryopreservation, because it can prevent spermatozoa from cold shock [4]. Egg yolks contain Low-Density Lipoprotein (LDL) which can bind to the BSP protein present in seminal plasma. This interaction will prevent the removal of fat from the plasma membrane and maintain spermatozoamotility during preservation [5]. The cholesterol content of each poultry egg is different [6] [7]. The difference in cholesterol in each quail egg yolk, broiler chicken and the duck is important to do research on the effect of giving different egg yolks to the quality of spermatozoa in the dilution of frozen semen.

The purpose of this study was to see the quality of Simental cow semen on tris diluents with different egg yolks.

2. Materials and methods

2.1. Semen collection

This study was carried out at the Tuah Sakato Artificial Insemination center, Payakumbuh, West Sumatera. One Simental bulls, aged at 5 years old, were used in this study. The Simental bulls were reared with management under the standard procedur in the Tuah Sakato artificial insemination center. The semen collection was done once a week for ten weeks by using an artificial vagina (Kruuse, model 340284, Denmark).

The semen that has been accommodated is evaluated macroscopically and microscopically. Macroscopically spermatozoa are assessed based on color, consistency, pH and volume. Microscopically spermatozoa are assessed based on the mass movement, spermatozoa motility, spermatozoa individual movements or individual scores, spermatozoa concentration, the viability of spermatozoa and plasma membrane integrity. Fresh semen showing spermatozoa motility> 70% with a concentration of more than 1000 million/mL, was used in the study.

2.2. Preparation of diluent

Tris was used as a buffer for the experimental diluent. It consisted of 3.028 g of tris-(hydroxymethyl)-aminomethane crystals, 1.25 g fructose crystals and 1.7 g citric monohydric acid in 73 ml distilled water. The water pH was 7.0 and the osmotic pressure 320 mOsmol kg^{-1}. Glycerol 6 % (Procedure standard at Tuah Sakato artificial insemination center) were added. Antibiotic combination; streptomycin 0.4 ml,penicillin- 0.5ml were added to the diluent. All chemical substances used are derived from Sigma Chemical Co. (St. Louis, Mo, USA). Egg yolk either from chicken, duck and equail was added at the rate of 20% (vol/vol) [8]

The tools used in this study are an artificial vagina, microscope, Erlenmeyer tube, measuring cup, digital scale, magnetic stirrer, pH paper, micropipette.

2.3. Semen processing

The semen is divided into three parts, each diluted with Tris egg yolk Chicken (TEYC), Tris egg yolk Duck (TEYD) and Tris Egg yolk Quail (TEYQ) diluents. Semen is diluted with a dilution dose of 100 million cells per mL (25 million cells/straw), then packaged into a mini straw. Spermatozoa were then packaged at a final the concentration of 25 x 10^6 sperm/mL in 0.25 mL straws (IMV Tecnologies, L'Aigle, Cedex, France) and sealed with polyvinyl alcohol. After the semen is packed, the semen is then relaxed at a temperature of 5 ^oC for 4 hours. The straws were kept in liquid nitrogen vapors for 10 min and then plunged into liquid nitrogen (-196^{0}C) for storage. After 24 h, the straws were thawed at 37 ^{0}C for at least 30 s in water bath. then the mini-straw plugs are cut and inserted into a microtube and then evaluated under a microscope to determine motility values, abnormality, viability, plasma membrane integrity, and recovery rate after freezing of spermatozoa

2.4. Parameters

Motility. A 5 µl drop of thawed semen was placed on a warmed (37 ^oC) glass slide and cover-slipped. And the examined under phase contrast a microscope (x400). The assessment was carried out by counting spermatozoathat moved forward compared to the total spermatozoa calculated in 200 sperm. The numbers given range from 0-100% with a scale of 5% [1].

Viability. Live spermatozoais calculated by laying one drop of semen that has been diluted on the glass of the object, added two drops of Eosin-nigrosin, stirred and made spread with the other end of the glass object along with the glass of the object. Screw preparation is dried and observed under phase contrast a microscope with 400x magnification. red spermatozoais living spermatozoaand white spermatozoameans dead sperm. Live spermatozoacounts compared to a minimum of 200 spermatozoacounts [1].

Abnormality. Semen sample (100µl), was fixed in 500 µl of 1% formal citrate. Spermatozoa per extender were examined using phase contrast microscope. Calculated abnormalities of the number of spermatozoa do not have a head without a tail, a crooked tail or a branched tail compared to the number of spermatozoa counted at least 200 spermatozoa[1].

Plasma membrane intact (PMI). Observation of intact plasma membrane of spermatozoa is done by dripping a drop of a mixture of spermatozoa(a mixture of spermatozoaand Host solution that has been incubated) on the glass object and covered with a glass cover, then observed under a microscope with

x400 magnification. Spermatozoa with a membrane that is still intact will hold hypoosmotic fluid in the cell, so that the tail looks circular or bent, while the spermatozoa with a straight tail show the plasma membrane has been damaged because it is unable to hold the incoming water [9].

Recovery rate. The percentage of recovery rate (RR) is calculated by looking at the percentage of motile spermatozoa after thawing divided by fresh spermatozoa motility multiplied by 100 [1].

2.5. Data analysis

Data collected was analyzed using a completely randomized design (CRD) of three treatments with ten replications. The difference in the mean value of the treatment was tested by the DMRT test [10].

3. Results and discussion

3.1. The Quality of fresh semen Simental bulls

The quality of Simental cattle fresh semen in this study showed that Simental beef semen was of good quality and feasible to use (Table 1). This quality is suitable for further dilution according to [11] which states that the condition of fresh semen can be diluted if it has a motility value of> 60%. Furthermore, the range of motility of 70-80% is the Semen that is worth diluting [1].

Table 1. The Quality of fresh semen Simental bulls

Parameters	Related
Volume (ml)	6
pH	7.0
Color	Cream whites
Consistency	medium
Smell	Spesifik
Kosentrasi	1.800
Mass motion	+++
Individual motion	+3
Motility (%)	75.00
The viability (%)	82.00
Abnormality (%)	7.50
The integrity plasma membrane (%)	80

3.2. Quality of Spermatozoa in Simental Cattle Post-Thawing

Motility.

Percentage of Simental spermatozoa motility after thawing on egg yolk treatment was significantly different from the use of different egg yolks. The motility of Simental spermatozoa on the use of egg yolks ducks (43%) was higher than the use of egg yolks quail (40.50%) and egg yolks chicken (38.67%) (Table 2).

Tabel 2. The average quality of post-thawing Simental bulls spermatozoa

Treatment	% Motility	PMI (%)	Viability	Abnormality	Recovery rate
Tris egg yolks chicken	38,67±1,21^{c}	48,67±1,03^{c}	37,50±1,87^{c}	24,00±2,76^{a}	48,67±1,03^{c}
Tris egg yolks duck	43,00±0,89^{a}	54,67±1,03^{a}	45,17±1,83^{a}	13,33±2,34^{c}	54,67±1,03^{a}
Tris egg yolks equil	40,50±1,05^{b}	50,50±1,87^{b}	42,50±2,26^{b}	18,00±2,28^{b}	50,50±1,87^{b}

note: Different superscripts in the same column show very real differences (P<0,01).

This difference is probably due to the cholesterol content in different egg yolks. According to the Polat *et al* [6] [7], each egg yolk in poultry produces a different amount of cholesterol. Egg yolk duck (884 mg) has the highest cholesterol content compared to egg yolk quail (844 mg) and egg yolk chicken (423 mg). Diluent which has a higher cholesterol content will have spermatozoa with plasma

membrane structure that is more compact and tends to be more resistant to the effects of cold shock [12].

A stable plasma membrane structure in spermatozoa, especially in the tail, will maintain the work of the aspartate aminotransferase enzyme in over-hauling energy from ATP to ADP and ADP to AMP. This reshuffle will cause a contraction of fibrils in the principle of peace and end piece. This contraction will cause spermatozoato move forward which is called motile [13]. Furthermore, Rizal [13] stated that if there is damage to the membrane, it can cause the loss of enzymes needed in the metabolic process so that energy is not produced, the motility will be low, and life force will also below.

The percentage of motility of spermatozoa after thawing ranged from 38.67 to 43.00% (Tabel 2). The results of the post-thawing examination showed that the percentage of motility was still quite good with an average of 40%. These results are still in accordance with the Indonesian frozen semen production standards listed in [11] which states that the quality of semen of cattle after undergoing a post-thawing process should show a minimum of 40% live and progressive motile spermatozoa.

The value of Simental semen motility in this study (40.5%) was lower than Simental bulls semen analyzed in Aceh (53.26%) [15]. This difference is caused by age, type of diluent used, handling of livestock and animal body weight [16].

Abnormality.

The mean abnormalities of spermatozoa after thawing ranged from 13.33 to 24.00% (Table 2). The percentage of Simental cow spermatozoa abnormalities in egg yolk originating from ducks was lower than the egg yolk from quails (18.00%) and egg yolk from broiler chickens (24.00%). This difference is caused by differences in egg yolks used in diluents. cooling causes an increase in abnormalities and damage to spermatozoa cells but can still be overcome by dilution containing egg yolk, because in the yolk there is lecithin and lipoprotein which function to protect and maintain the integrity of the spermatozoa cell lipoprotein sheath and prevent cold grip [4].

Lipoprotein will protect from outside the cell by putting itself on the plasma membrane so that spermatozoa is encased by lipoprotein. Lipoprotein is the main component in the egg yolk that protects the spermatozoa plasma membrane [8]. The percentage of abnormalities is still said to be good because of the average ranges below 20%. This is also according to opinion [11], who said that the morphological abnormalities of spermatozoa below 20% were still considered normal. Abnormalities of spermatozoa can occur in the head and tail of the spermatozoa and can occur during the process of spermatogenesis or after the outflow of the epididymal duct [17]. The percentage of abnormalities of Simental bulls spermatozoa after thawing in this study was higher (13.33%) compared to Boer goat spermatozoa in Tris diluent using egg yolk duck (4.4%) [18]. This difference is likely due to differences in livestock types and dilution techniques used [19].

Viability

The viability of Simental bulls post thawing spermatozoa was higher in the use of tris egg yolk duck (45.17%) compared to the use of tris egg yolk Quail (42.50%) and tris egg yolk Chicken (37.50%) (Table 2). This difference is caused by differences in the composition of cholesterol in egg yolks used in diluents. The content of Low-density lipoprotein (LDL) in egg yolk, especially phospholipids which have been identified as an effective component in protecting spermatozoa from the effects of rapid cooling [20].

The viability of spermatozoa decreases due to plasma membrane damage due to the influence of cold shock and the viability of living spermatozoa is affected by the integrity of the plasma membrane [4]. The decrease in the value of semen motility during post thawing is very much due to differences in membrane conditions during freezing and the process of thawing. The freezing process forms ice crystals and electrolyte and other dissolved materials occur. Excessive electrolyte concentration will dissolve the lipoprotein sheath of the spermatozoa wall and the time of thawing again permeability of the cell membrane will change and cause spermatozoa cell death [13]. The viability of spermatozoa is higher than motility, this is because spermatozoa that lives is not necessarily able to move, but immobile spermatozoa is sometimes still alive [17].

The viability of Simental bulls spermatozoa in this study is different from Simental spermatozoa in Sulawesi with the dilution of tris fructose egg yolk chicken (37%-45% VS 61%-67%) [21]. This difference is due to different dilution techniques and different fresh semen motility values [1].

The membrane plasma intact

The highest percentage of the plasma membrane intact in post-thawing Simental bulls spermatozoa was found in tris egg yolk duck (54.67%), followed by the percentage of PMI in tris egg yolk quail (50.50%) and the lowest in tris egg yolk chicken (48.67%). Analysis of the composition of various types of egg yolk shows that the basic components of the yolk are similar, but the ratio of fatty acids and phospholipid class is different [22]. furthermore, Bathgate *et al* [20], explained that duck yolk has more monounsaturated fatty acids (MUFA) than chicken egg yolk, and quail egg yolk. Duck Egg yolk contains more phosphatidylinositol (PI) than egg yolk chicken or egg yolk quail and egg yolk quail contain more phosphatidylserine than chicken egg yolk or ducks egg yolk.

An intact plasma membrane will hold osmolality fluid in the cell so that the tail looks circular or bent while spermatozoa with a straight tail shows that the plasma membrane has suffered damage because it is unable to hold the liquid entering the cell. Damage to the plasma membrane will affect the metabolic process and is related to the motility and viability of the spermatozoa produced. Cell metabolism will take place well if the plasma membrane of the cell is in an intact state so that it can properly regulate traffic in and out of the cells of all the substrates and electrolytes needed in the metabolic process[23]. Good spermatozoa membrane integrity shows that phospholipids can survive and maintain well when freezing and thawing again. Phospholipids function to maintain membrane integrity and form a dynamic surface between cells as protection against environmental conditions [17].

Recovery Rate

The percentage of recovery rate (RR) Simental spermatozoa after thawing was highest in duck egg yolk (57.33%), followed by RR percentage in quail egg yolk (54.00%) and the lowest in tris egg yolk chicken (51.56%). This difference is caused by Low-Density Lipoprotein (LDL) egg yolks used in different diluent materials [19]. This is because LDL will bind to the cell plasma membrane phospholipid which causes the cell plasma membrane to be more flexible so that it can cope with cold stresses during the dilution and storage process [5].

High RR values indicate that spermatozoa have high resistance after the freezing process while low RR values indicate that spermatozoa have low endurance [1]. The loss of motility of the spermatozoa during the freezing process will affect the recovery rate (RR) of the spermatozoa after thawing. The RR value in this study is different from the percentage RR of Frisian Holand bulls (59.4% -69.56%)[24] and Percentage of RR spermatozoa Limousine (44% -62%) [25]. This difference is caused by the value of fresh spermatozoa motility and the motility value after thawing that is different between livestock. This is according to Akal *et al* [4] that the high and low RR values are caused by the value of motility, individual livestock, dilution, and freezing.

4. Conclusions

The addition of duck egg yolk to the tris diluent for Simental bulls spermatozoa preservation is the best diluent with high motility (43.00%), high intact plasma membrane (54.67%), high the viability (45.17%) with low abnormality spermatozoa (13.33%) and lower recovery rate (57.33 %) compared to the tris egg yolk-chicken diluent and tris egg yolk-quail.

Recomendation

It is recommended to use duck egg yolk in the tris diluent for preserved Simental bull spermatozoa

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